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Review

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The Era of Multigene Panels Comes? The Clinical Utility of Oncotype DX and MammaPrint

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Abstract

The AJCC Cancer Staging Manual, eighth edition published in late 2016, will become the new global guideline for cancer diagnosis and treatment from January 1, 2018. The new edition for the tumor staging system has numerous updates, including building up the prognostic stage group of tumors for the first time and adding a large number of non-anatomical factors into the prognostic evaluation. Oncotype DX and MammaPrint are two of the genomic predictors that will be part of routine clinical practice in the future. Numerous studies have proved the clinical utility of multigene panels in predicting clinical outcome and treatment response. Here we present our review of the studies on these multigene panels and their application to breast cancer.

Keywords: Breast cancer; Multigene panel; Oncotype DX; MammaPrint

Introduction

In 2013, the 13th St Gallen Panel recommended that gene expression panels be used to identify patients with good clinical outcome, which affirmed the value of multigene assays in clinical management among patients of luminal type breast cancer [1]. Two available tests, Oncotype DX and MammaPrint, have exhibited good metric performances in the prediction of prognosis and treatment benefit of early-stage breast cancer and these genomic predictors are in routine clinical practice in some areas. Multigene expression-based assays used to analyze an array of prognostic biomarkers have been shown to help direct treatment decisions, which have become part of the AJCC Prognostic Stage Group described by AJCC Cancer Stage Manual, the eighth edition in late 2016 [2]. We will review Oncotype DX and MammaPrint to discuss their current status and limitation in clinical practice.

Oncotype DX (Recurrence Score (RS))

Oncotype DX is performed on RNA extracted from formalin-fixed paraffin-embedded tumor tissue, using quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR). It contains a set of five reference genes (ACTB, GAPDH, GUS, RPLPO and TFRC) and 16 cancer-related genes. The RS is the result of a mathematical formula of the weighted expression of each gene, ranging from 0 to 100. The cutoff points are divided into three categories: low risk (RS < 18), intermediate risk (RS 18 - 30), and high risk (RS > 31). The RS has been proved to be a predictor of 10-year distant recurrence for early breast cancer through NSABP B-14 in multivariate analyses including age, tumor size, tumor grade, estrogen receptor (ER) status and HER2 status [3]. Furthermore, patients with low or intermediate RS had large improvements in disease-free survival (DFS) if treated with tamoxifen (TAM), which indicated that RS was helpful in evaluating treatment response to endocrine therapy in early breast cancer. Habel et al [4] conducted a case-control study among women with ER-positive, node-negative breast cancer treated with TAM and compared these with untreated patients. The RS was associated with the risk of breast cancer death in both groups ($P = 0.003$ and $P = 0.03$). Thus, the RS was strongly related to long-term mortality of breast cancer among ER-positive breast cancer patients treated with endocrine therapy.

Paik et al [3] not only evaluated the relationship between the RS and clinical result of ER-positive, node-negative early breast cancer, but also explored the prognostic ability in late recurrence of breast cancer. The 10-year distant recurrence rates among low-, intermediate- and high-risk groups were 6.8%, 14.3% and 30.5%, respectively. It showed that the RS was related to distant relapse in patients without adjuvant chemotherapy, regardless of age and tumor size and performed better than both of them ($P < 0.001$). A summary of key studies can be found in Table 1 [3, 5-13].

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Table 1. Key Studies of Clinical Evidence to the Oncotype DX [3, 5-13]

Study	Prospective trial	Reported by	N	Patient population	Treatment	End point	Result
NSABP B-14 [3]	Yes	Paik et al	668	HR^+, node-negative	TAM	10-year distant recurrence/ secondary end point: RS and risk of distant recurrence	The rates of 10-year distant recurrence were 6.8%, 14.3% and 30.5% in low-risk, intermediate-risk and high-risk group.
WGS Plan B [10]	Yes	Gluz et al	3,198	HR^+, 41.1% node-positive	Endocrine therapy and chemotherapy, endocrine alone if RS ≤ 11	DFS	The 3-year DFS is quiet low in patients omitted with chemotherapy with RS ≤ 11.
PACS 01 trial [9]	Yes	Penault-Llorca et al	530	HR^+, node-positive	FEC for six cycles or FEC for three cycles followed by docetaxel for three cycles	DRFI/second end point: DFS and OS	The 5-year DRFI of low risk of RS is 93.7% versus 87.3% and 69.3% in intermediate or high RS ($P < 0.001$). The 21-gene RS maintains significant prognostic impact in HR^+, node-positive pts who have received FEC or FEC-D adjuvant chemotherapy.
TAILORx [6]	Yes	Sparano et al	1,626	HR^+, $HER2^-$, node-negative, cT1cT2 or cT1b with high tumor grade	Endocrine therapy (TAM or anastrozole)	DFS/secondary end point: DDFS,OS	Among patients with tumors that had a favorable gene-expression panel had very low rates of recurrence at 5 years with endocrine therapy alone.
NSABP B-20 [5]	Yes	Paik et al	651	ER^+, node-negative	TAM or TAM plus chemotherapy	DFS	Patients with high-RS tumors had a large benefit from chemotherapy, 10-year distant recurrence rate decreased by 27.6%. Patients with low-RS tumors derived minimal benefit from chemotherapy.
SWOG-8814 [7]	Retrospective analysis for RS	Albain et al	367	HR^+, node-positive	TAM or CAF followed by TAM	DFS/OS	There was no benefit of CAF in patients with a low recurrence score (score < 18; $P = 0.97$), but an improvement in disease-free survival for those with a high recurrence score (score ≥ 31; $P = 0.033$), after adjustment for number of positive nodes.
ECOG E2197 [12]	Yes	Esteva et al	465	HR^+, 0 - 3 node-positive	AC for four cycles or TA for cycles	Recurrence free interval (RFI)	RS was a highly significant predictor of recurrence. The 5-year recurrence rate was less than 5% or for the estimated 46% of patients who have a low RS (< 18).
TranATAC [13]	Yes	Dowsett et al	1,071	HR^+, postmenopausal women	Endocrine therapy: tamoxifen, anastrozole or combination	Distant recurrence free survival/OS	The 9-year DR rates in low, intermediate, and high RS groups were 4%, 12%, and 25% in N0 patients; 17%, 28%, and 49%, in N+ patients. The prognostic value of RS was similar in anastrozole- and tamoxifen-treated patients.
NSABP B-28 [8]	Yes	Solin et al	1,065	ER^+, operable breast cancer node-positive	AC × 4 or AC × 4 followed by paclitaxel × 4 endocrine therapy	LRR as first event, DFS, OS	The 10-year LRR for low, intermediate, high RS is 3.3%, 7.2% and 12.2%. RS statistically significantly predict risk of LRR in node-positive, ER-positive breast cancer patients after chemotherapy and endocrine therapy.
RxPONDER [11]	Yes	Ramsey et al	Ongoing	HR^+, $HER2^-$, 1 - 3 lymph nodes and RS ≤ 25	Endocrine therapy alone versus chemotherapy followed by endocrine therapy	DFS and cutoff point for RS value, secondary endpoint: DDFS, local disease-free interval, OS	Ongoing, intend to report in 2022.

The Clinical Utility of Oncotype DX (RS)

RS can predict chemotherapy sensitivity in patients with ER-positive, node-negative breast cancer. Paik et al [5] studied 651 cases of breast cancer who were enrolled in NSABP B-20 and randomly assigned them into a TAM group and a TAM combined with chemotherapy group (chemotherapy regimen for CMF or MF regimen, TAM + CMF/MF group). The 10-year follow-up results showed that patients with high RS had benefited from cytotoxic chemotherapy, with the 10-year metastasis rate being decreased by 27.6%. In contrast, the 10-year distant metastasis rate was decreased by an average of -1.1% in patients with low RS who received adjuvant chemotherapy. Therefore, patients with ER-positive early breast cancer and high RS should benefit from chemotherapy, whilst patients with low RS cannot. RS can help select patients who experience little benefit of chemotherapy and can avoid the toxic effects of chemotherapy.

In a phase III trial, the Trial Assigning Individualized Options for Treatment (TAILORx), there was a prospective phase to further validate the function of RS in patients with hormone-receptor (HR)-positive, HER2-negative, node-negative breast cancer. The results from TAILORx indicated that patients with very low RS results (< 11) had excellent clinical outcome with a rate of 5-year freedom from distant recurrence with endocrine therapy at 99.3% and a rate of overall survival (OS) of 98.0%, even without chemotherapy [6]. As for its excellent utility in identifying patients with good outcome, Oncotype DX RS became the only gene-expression assay that was recommended at level I evidence in the AJCC Prognostic Stage Group.

In patients with HR-positive, node-negative breast cancer, the RS showed excellent clinical utility to predict clinical outcomes. The Southwest Oncology Group (SWOG)-8814 focused on exploring the benefit of therapy in patients with HR-positive, node-positive breast cancer. It enrolled postmenopausal women treated with chemotherapy or simple endocrine adjuvant therapy, of which 367 cases (40%) received an RS detection. The results showed that RS had a definite predictive value (P = 0.016) for adjuvant treatment benefit over 5 years and was poorly predicted for treatment beyond 5 years (P = 0.87). High-risk patients receiving chemotherapy combined with endocrine therapy compared with simple endocrine therapy benefit significantly (P = 0.033). SWOG-8814 trial showed that the RS was also prognostic for TAM-treated patients with positive nodes and predicts significant benefit of chemotherapy (CMF) in tumors with a high RS [7].

ECOG E2197 also studied the predictive utility of RS on loco-regional recurrence (LRR) in 388 lumpectomy patients with 0 - 3 positive nodes treated with chemo-endocrine therapy and breast radiology. With 9.7 years median follow-up, the 10-year rates of LRR for HR-positive tumors were 3.8%, 5.1% and 12.0% for low, intermediate and high risk of RS (P = 0.12) [14]. Similarly, in NSABP B-28, which compared the benefit to patients from chemotherapy and endocrine therapy, RS was a statistically predictor of LRR, with 10-year cumulative incidence of LRR of 3.3%, 7.2% and 12.2% in low, intermediate and high RS (P < 0.001) [8]. RS is a strongly predictive factor of LRR for HR-positive breast cancer regardless of node status. Another study, PACS 01 trial ,with a median of 7.7 years follow-up, showed that RS was a significant predictor of DRFIS, DFS and OS (P < 0.001) in HR-positive, node-positive patients treated with chemotherapy plus endocrine therapy [9].

In a recent prospective phase III trial, West German Study Group Plan B, 348 patients (15.8%) with RS ≤ 11 had excellent 3-year survival even if they omitted chemotherapy. The 3-year DFS in patients with RS ≤ 11 was 98%, in which 41.1% had node-positive and 32.5% were grade 3 disease. These were the first prospective data to report clinical outcome when RS was used to make physical decision in patients with HR-positive breast cancer regardless of lymph node invasion [10].

Another ongoing multicenter phase III trial, RxPONDER trial (Rx for Positive Node, Endocrine Responsive Breast Cancer) revealed that patients with node positive breast cancer who had low to intermediate RS results could benefit from chemotherapy [11]. The trial also determined whether there is an optimal RS cutoff for these patients above which chemotherapy should be recommended in clinical practice. RxPONDER trial randomized patients with HR-positive, HER2-negative and 1 - 3 lymph nodes breast cancer with RS ≤ 25, to improve the risk of stratification in patients with low or intermediate RS.

MammaPrint

MammaPrint was first developed by the Netherlands Cancer Institute group. Van't Veer et al [15] used a gene-expression panel to detect 78 frozen tumor tissues from patients with pT1-2cN0 invasive breast carcinoma who had received standard treatment. Ribonucleic acid (RNA) was isolated from fresh frozen tumor tissue to obtain complementary DNA (cDNA). The gene-expression panel contains 70 genes related to early risk of metastasis, including tumor invasion, metastasis, interstitial invasion, and angiogenesis-related genes. A total of 295 patients under 53 years old were enrolled in the study. The patient had tumors that were of different tumor types as assessed using immunohistochemistry results and lymph node status. All the patients were assessed to follow up for at least 5 years. From the gene expression, patients were divided into good prognosis signature and poor prognosis signature. The results showed risk of 10-year distant metastasis in patients of poor-prognosis signature was significantly higher than that in good-prognosis signature (85% versus 51%, P < 0.001) and overall 10-year survival was 95% versus 55% in these two groups. Compared with histological grade, primary tumor size, vascular invasion, hormone receptor status and other adverse factors, the 70-gene panel was the strongest predictor of disease progression [16]. In addition, patients in the poor-prognosis group who received chemotherapy had a significantly reduced 10-year distant metastasis rate (HR 0.37, P < 0.001). Therefore, it is recommended that patients with poor-prognosis signature receive cytotoxic adjuvant chemotherapy in order to reduce the risk of distant metastasis [12].

The Clinical Utility of MammaPrint

The microarray-prognostics-in-breast-cancer (RASTER) trial

was the first prospective phase III clinical trial to investigate the prognostic value of the 70-gene signature for distant recurrence in patients with breast cancer and also compared the 70-gene signature with the Adjuvant! Online (AOL). A total of 427 cases of cT1-3N0M0 breast cancer patients were enrolled. Both the 70-gene expression and AOL were taken into consideration whilst making adjuvant systemic treatment decision. With a median follow-up of 61.6 months, 5-year DRFI probabilities for 70-gene signature low-risk AOL high-risk patients were 98.4%, in which 76% had not received adjuvant chemotherapy. The results indicated that the 70-gene signature low-risk predicted low-risk rate in 5-year distant metastasis/good clinical result regardless of AOL score. Compared with AOL, the 70-gene signature is more valuable for patients with good clinical outcome [17].

Other studies aiming at investigating MammaPrint validation in patients with node-positive breast cancer have been conducted. Mook et al [18] further investigated whether the 70-gene prognosis signature could predict the clinical outcome for breast cancer patients with lymph node invasion. Two hundred forty-one patients with T1-3N1M0 breast cancer were selected to conduct the 70-gene signature and received standard treatment including surgery followed by systemic adjuvant therapy and radiotherapy. Ninty-nine patients (41%) were assigned to the good-prognosis group, and 142 cases (59%) to the poor-diagnosis group. The rates of 10-year distant metastasis-free survival and breast cancer specific survival were significantly higher in the good-prognosis group than the poor-prognosis group (91% versus 76%, 96% versus 76%). The results of multivariate analysis suggested that the predictive value of the 70-gene prognosis signature was significantly superior to lymph node invasion, histological grade and ER status in predicting breast cancer outcome. It is suggested that the 70-gene prognosis signature can predict the disease outcome in patients with node-positive breast cancer.

A randomized prospective trial, the MINDACT study, recently further confirmed the clinical utility of MammaPrint. MINDACT involved 6,693 new diagnosed breast cancer patients with 0 - 3 metastatic lymph nodes [19]. After 5 years median follow-up, the study demonstrated excellent clinical outcomes in patients at high clinical risk but with low genomic risk even omitting chemotherapy. Of all patients with breast cancer at the highest clinical risk, 46% were categorized as being low genomic risk. The distant metastasis-free survival was 94.7% in these patients at low genomic risk without chemotherapy, 1.5% age points lower compared to patients treated with chemotherapy. This indicated that 46% of patients with breast cancer at a high clinical risk might not require chemotherapy because of low risk as evaluated by MammaPrint. The 70-gene prognosis signature outperforms traditional prognostic factors in predicting disease outcome in patients with 0 - 3 positive nodes. Moreover, the signature can accurately identify patients with an excellent disease outcome in patients who may be safely spared adjuvant chemotherapy. For this reason, EGTM (The European Group on Tumour Markers) recommended MammaPrint to be used for determining prognosis and guiding decision-making with respect to the administration of adjuvant chemotherapy in patients with newly diagnosed invasive breast cancer that is lymph node negative or 1- 3 lymph node positive. Patients with high clinical risk but low risk based on MammaPrint can avoid adjuvant chemotherapy [20]. MammaPrint also became the first assay to be cleared at the 510(k) level approved by the US FDA [21], used to predict the clinical outcome of women under the age of 61 years with ER-positive or negative, lymph node-negative breast cancer [22].

Relationship Among Different Gene Panels

Fan et al [23] compared the prognostic value of five gene-expression panels including intrinsic subtypes, the 70-gene prognosis signature (MammaPrint), wound response, RS (Oncotype Dx) and the two-gene ratio. The models based on intrinsic subtypes, the 70-gene signature, wound response and RS were significant predictors of relapse-free survival and OS. They then compared RS and MammaPrint in a group of 295 patients, considering low and intermediate RS with good-prognosis signature in the 70-gene signature. Eighty-one percent of samples agreed in the two models, especially in patients with good clinical outcome. In addition, $HER2^+$ and ER-negative subtype and poor-outcome luminal B subtype were likely to be in the poor-prognosis groups in both of 70-gene prognosis signature and RS. After that trial, Prat et al [24] compared the prognostic value of the PAM50 intrinsic subtyping and risk of RS, the 70-gene signature, relapse (PAM50-ROR) score, Rotterdam 76 gene and the index of sensitivity to endocrine therapy (SET) in 594 patients who received local treatment and TAM only. The result confirmed that RS and the 70-gene prognosis signature were highly prognostic in breast cancer patients in disease relapse risk from the genetic level, whilst instinct subtypes distinguish patients of different risk from molecular or protein expression level. Although the genes detected and the techniques differ between RS and MammaPrint, both of the two gene panels are proved to be consistent with long-term clinical outcome, especially accurately identifying breast cancer patients with a low risk of recurrence who are predicted to derive minimal benefit from chemotherapy.

Present and Prospective for Multigene Panels

In the last decade, clinicians and researchers have been dedicated in trying to solve how to avoid under and overtreatment in patients with curable breast cancer. The ability to personalize treatment based on tumor biology and to reduce unnecessary chemotherapy is the key to optimizing patient care. Gene panels have proved to be promising tumor markers in predicting disease progress and making personalized physical decision [25]. After TAILORx and many other prospective trials, RS and the 70-gene prognosis signature have been proved to have clinical relevance with disease outcome. The two gene panels are most well validated and used in the clinical setting to assist in treatment decisions for patients with HR-positive early stage breast cancer.

Many international guidelines have affirmed the clinical utility of gene panels. These studies were discussed in the recent St Gallen International Expert Panel Meetings (2017) on

the primary therapy of early breast cancer, seeking consensus as to the instances that multigene panels could be used to assist physician decision. The majority of the panel agreed that for patients with ER^+, $HER2^-$ breast cancer, clinically valuable information on prognosis and risk helping to omit chemotherapy may be provided by RS and MammaPrint both in patients of node-negative or positive breast cancer. Both Oncotype DX and MammaPrint can predict distant recurrence in at least 5 years; however, the efficacy of multigene panels in predicting relapse rates in HR-positive disease beyond 5 years remains to be confirmed by further studies.

The current use of multigene panels in clinical practice does reflect a personalized clinical decision-making progress. Although the cost of RS and the 70-gene prognosis signature is currently $3,460 and $4,250, respectively, a number of retrospective studies have confirmed the cost-effectiveness of gene panels in treatment decision-making of breast cancer. Each patient benefits from £3,000 to £40,000 per quality-adjusted life year, indicating that the application of gene panels in clinical practice avoids overtreatment as well as reducing treatment cost [26, 27]. Loncaster et al [28] evaluated the impact of RS on adjuvant chemotherapy decision-making in clinical practice in patients with early curable breast cancer and showed that 60.3% of ER^+, $HER2^-$, node-negative patients were spared chemotherapy due to low RS. Even in node-positive patients, many avoided adjuvant chemotherapy as well. A meta-analysis containing 2,229 patients showed 29.52% treatment decisions on chemotherapy changed due to the use of RS, of which 12% are reduction of chemotherapy [29]. Similarly, Exner et al [30] reported that decision changes were recorded in 18.6% cases and that two-thirds of patients can be spared chemotherapy due to the use of MammaPrint. The routine use of multigene panels in early breast cancer has significant cost saving [25]. Currently, RS is recommended in patients with ER^+, $HER2^-$, $T > 0.5$ cm early breast cancer by the National Comprehensive Cancer Network (NCCN), the European Society for Medical Oncology (ESMO) ,the American Society of Clinical Oncology (ASCO), the St Gallen Group and the EGTM, Arbeitsgemeinschaft Gynakologische Onkologie (AGO) for use in selected patients [1, 20, 27, 28, 31, 32]. It is also recommended for patients with node-positive breast cancer using the guidelines above, except the ASCO guideline. MammaPrint is recommended for patients with node-positive breast cancer in ESMO, St Gallen and EGTM, but not in ASCO and NCCN guideline [20].

Due to the multigene panel including genes related to biological behavior such as cancer metastasis and invasion, the clinical utility of multigene panels will be expanded as studies continue. Jegadeesh et al found RS > 24 was related with LRR in patients who received mastectomy and that the 5-year LRR rate was 27.3% versus 10.7% in patients of RS ≤ 24, indicating patients with RS > 24 may benefit from post-mastectomy [33]. The recently published report from the NSABP B-28 also showed that RS was a strong predictive factor of LRR in ER-positive, node-positive breast cancer after treatment; the final result will reveal how RS helps in selecting patients for comprehensive radiology, which may broaden the clinical utility of RS [34]. Similarly to NSABP B-28, MINDACT has more broadly represent women with breast cancer than other studies with MammaPrint. In MINDACT, 10% of enrolled patients were HER2-positive, 12% were HR-negative, and 20% had 1 - 3 positive nodes. The result of MINDACT can perhaps broaden the clinical utility of MammaPrint if the genetic tumor screening is able to find out who requires chemotherapy and who does not [35].

Limitation of Multigene Panels

The multigene panels represent a conceptual advance over arbitrary groupings based on traditional clinicopathlogical factors [36]. Studies on gene panels show a promising future for clinical use, although some limitations hinder the techniques from wider use. One such limitation is that the cutoff for gene panels varies between studies. The consistent findings of large prospective clinical trials further validate the clinical utility of the RS and strongly suggest that adjuvant chemotherapy may be spared for patients with very low (< 10) RS results [37]. We are seeking consensus on the evidence and opinions regarding correct cutoff points and how to deal with patients of intermediate risk identified by the RS. As Jiang, a member of the 15th St Gallen consensus panels mentioned in the voting session: if we use multigene assays in clinical practice, the first thing is to clearly understand how high the genomic score or risk is high. Another limitation is that MammaPrint requires freshly frozen tissue collected into an RNA preservative solution, which makes it more complex to conduct than other panels. Otherwise, disparities in access to genomic diagnostic test in some area or race also make it difficult to maximum the clinical utility of gene panels [38, 39].

Conclusion

Multigene panels can provide better risk discrimination relative to clinicopathological factors, which are significantly superior to traditional prognostic factors in predicting clinical outcome and identifying patients who can be spared chemotherapy safely. Available evidence supports the clinical validation of multigene panels and that they are proved to be cost saving while assisting treatment decision. Oncotype DX and MammaPrint have the strongest evidence supporting their clinical utility and decision effectiveness, and have been widely used in luminal type breast cancer [40]. The AJCC Prognostic Stage Group containing multigene panels will be globally used from January 1, 2018. It suggests that prognostic stage grouping should be used in countries where biomarker tests are routinely performed, indicating that multigene molecular profiling will become part of cancer stage evaluation, and will need to be taken into consideration when making clinical decisions [2]. The future of multigene panels is promising in personalizing treatment as more studies continue. However, many issues remain to be solved before multigene panels have a wider influence on breast cancer treatment. Importantly new issues, such as how to accurately predicate late recurrence in ER-positive cancer and how to provide more access to multigene panels, should be solved in the future.

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